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The combined use of AgNORs and oral mucosal keratinization for early prediction of cytological atypia associated with the use of smokeless tobacco

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ABSTRACT

Background: Oral use of smokeless tobacco has been documented as a prime cause of oral cancer. Thus, the present study aimed to evaluate the merit of combined use of AgNORs and oral mucosal keratinization for early prediction of cytological atypia associated with smokeless tobacco use. *Methodology*: This was a case-control study that included 96 participants, 48 persons were smokeless tobacco users ascertained as the case group, and 48 individuals were non-tobacco users affirmed as a control group. All study subjects were selected from the Hail area, Saudi Araba, from October 2019 to January 2020. Cytological materials were obtained from all participants by scraping of oral mucosa using a wooden tongue depressor. *Results*: Mean counts of AgNORs of > 2 dots were identified in 25/48(52%) of the cases and only one (2%) of the controls. Keratinization was detected in 33/48(67%) of the cases and 6/48(12.5%) of the controls. Out of the 25 cases with abnormal AgNORs count, 23/25(92%) were detected with keratinization. *Conclusion:* The combined use of AgNORs and oral mucosal keratinization is useful for the early prediction of cytological atypia associated with smokeless tobacco use. Oral use of smokeless can cause oral mucosal keratinization and cellular proliferation.

Keywords: AgNORs, oral mucosa, keratinization, smokeless tobacco

1. INTRODUCTION

Cancer of the oral cavity is prevalent cancer worldwide (Lingström and Simark, 2020). Increased risk of oral cancer has been attributed to many etiological factors, including viral infection (such as human papillomavirus), tobacco use, alcohol consumption, and other oral hygiene-related factors (Ahmed, 2013). Smokeless tobacco is used by over 350 million people worldwide and responsible for around 650000 deathsworldwide—most smokeless tobacco products containing high levels of carcinogenic nitrosamines (Gupta and Mehrotra, 2021). The most potent carcinogenic product found in smokeless tobacco is tobacco-specific nitrosamines (TSNAs) (Xia et al., 2021), a well-established cause of oral cancer (Ahmed and Mahgoob, 2007).

Oral cytopathology is regarded as a reliable tool for screening at-risk populations to detect the early oral mucosal proliferative activity before progression into neoplasia (Ahmed et al., 2020). The Argyrophilic (Ag) nucleolar organizer regions (NORs) (AgNORs) method is a cost-effective, reliable method for screening those exposed to carcinogens (e.g., TSNAs) (Ahmed et al., 2020). Moreover, the oral epithelium's keratinization was established as an indicator for the carcinogenic effects on the buccal mucosa (Miyazono et al., 2020). Therefore, the present study aimed to assess the merit of combined use of AgNORs and oral mucosal keratinization for early prediction of cytological atypia associated with the use of smokeless tobacco.

2. MATERIALS AND METHODS

This was a case-control study that included 96 volunteer contributors living in Hail, Northern Saudi Arabia, from October 2019 to January 2020. All study subjects were randomly selected for this study using a simple random method; 48 individuals were smokeless tobacco users ascertained as a case group, and 48 individuals were non-tobacco users documented as control groups. All the study subjects were males their ages between 20 and 55 years old. Cytological materials were obtained from all participants by scraping of oral mucosa using a wooden tongue depressor. The obtained materials were smeared in glass slides, fixed in 95% ethyl alcohol, then were sent to the histopathology laboratory at the College of Medicine, University of Ha'il. Two smears were prepared for the materials obtained from each individual. One smear was stained using the Papanicolaou method (Pap. Method), and the other was stained using the AgNORs method.

Ethical Consent

Each participant was asked to sign a written ethical consent before the interview to obtain the personnel identification data.

Data Analysis

Obtained data were analyzed using SPSS software. Chi-square and odds ratios (OR) were obtained.

3. RESULTS

Most study subjects were aged 20-29 years, followed by other age groups in ascending aging (figure 1).

The mean counts of AgNORs of > 2 dots were identified in 25/48(52%) of the cases and only one (2%) of the controls (microphotograph 1). The mean NORs for cases were 2.36 and 1.05 for controls. The risk associated with smokeless tobacco, the odds ratio (OR), and the 95% confidence interval (95%CI); OR (95%CI) = 51(6.511-400.9, P-value =0.0002. Keratinization was detected in 33/48(67%) of the cases and 6/48(12.5%) of the controls (microphotograph 2). The OR (95%CI) = 15.4(5.4-44.0445), P-value =0.0002, as indicated in Table 1 and Fig 2.

Out of the 25 cases with abnormal AgNORs counts, 23/25(92%) were detected with keratinization. AgNORs counts and keratinization were significantly correlated in the prediction of early oral cellular response induced smokeless tobacco use (P-value <0.0001).

As indicated in Table 2 and Fig 3, fungal infection was identified in 14(29%) cases compared to 4(8.3%) controls. Evidence of viral infection was demonstrated in 7(14.5%) cases, and 3(6.3%) controls. Evidence of bacterial infection was seen in 4(8.3%) cases, and 4(8.3%) controls. Inflammatory cell infiltrates were identified in 12(25%) cases and 2(4.2%) controls.

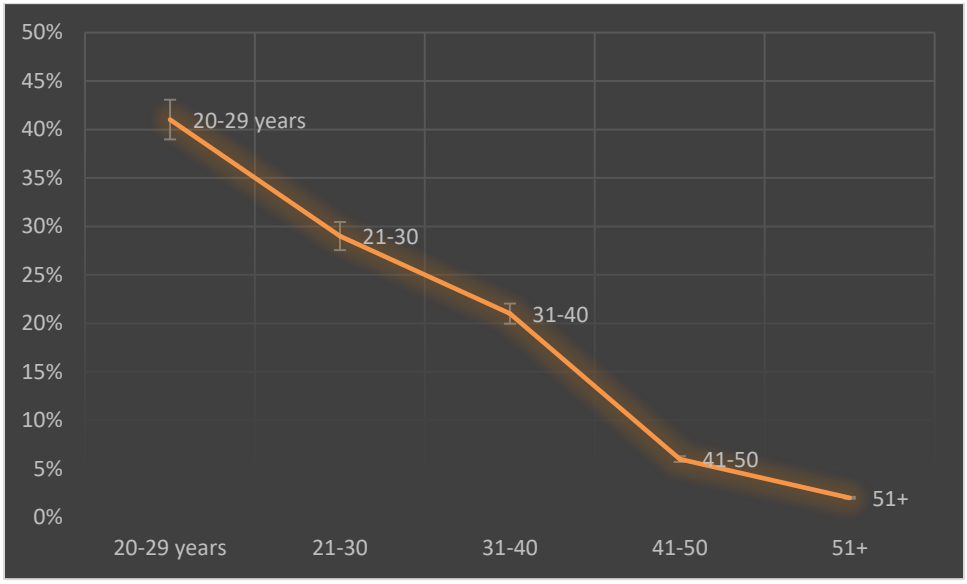


Figure 1 Study population by age

Table 1 Distribution of the cases and controls by AgNORs mean count and epithelium cells keratinization.

Variable	Cases	Controls	Total	OR	P-value
AgNORs mean dots					
>2 dots	25	1	26	51(6.511-400.9	0.0002
< 2 dots	23	47	70		
Total	48	48	96		
Keratinization					
Present	33	6	39	15.4(5.4-44.0445)	0.0001
Absent	15	42	57		
Total	48	48	96		

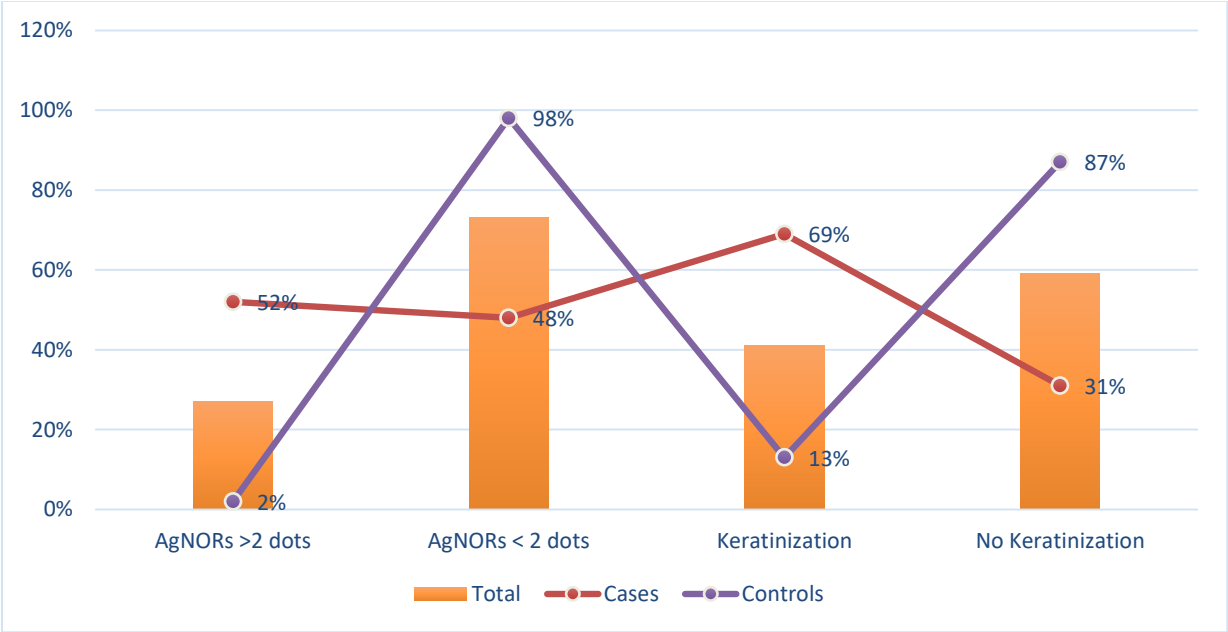
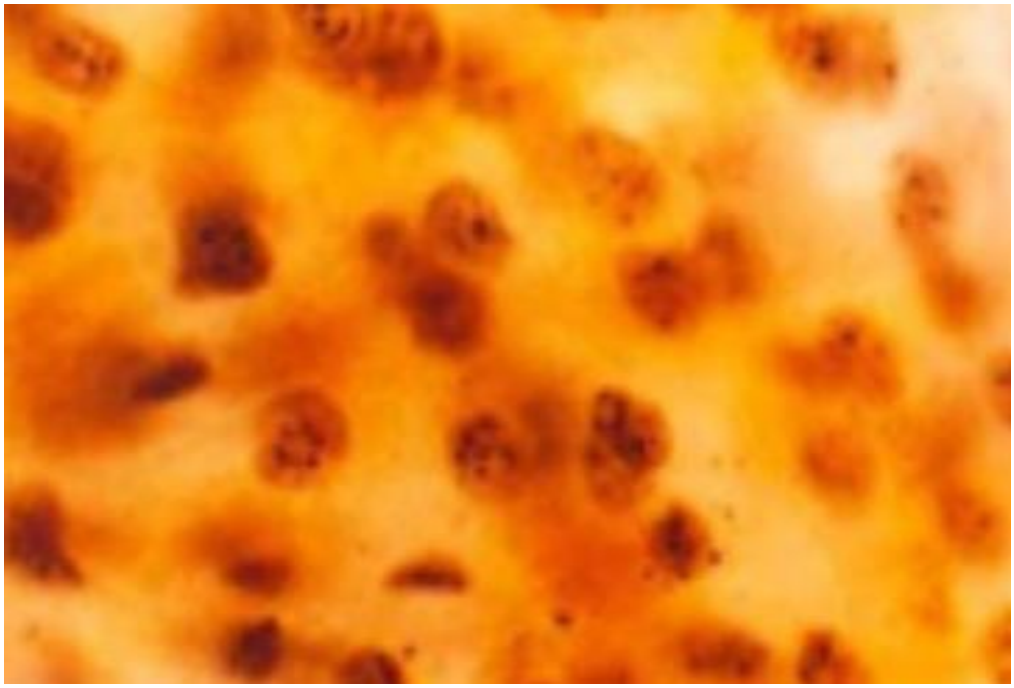


Figure 2 Description of the cases and controls by AgNORs and Keratinization



Microphotograph 1 Buccal smear obtained from smokeless tobacco users. AgNOR staining showing NORs dots. Magnification 400

Microphotograph 2 Buccal smear obtained from smokeless tobacco users. Pap. Stained smear showing excessive keratinization. Magnification 400.

Table 2 Distribution of the study subjects by infections and inflammatory cells infiltrate.

Variable	Cases	Controls	Total
Infections			
Fungal	14	4	18
Viral	7	3	10

Bacterial	4	4	8
Total	25	11	36
Inflammatory			
Present	12	2	14
absent	36	46	82
Total	48	48	96

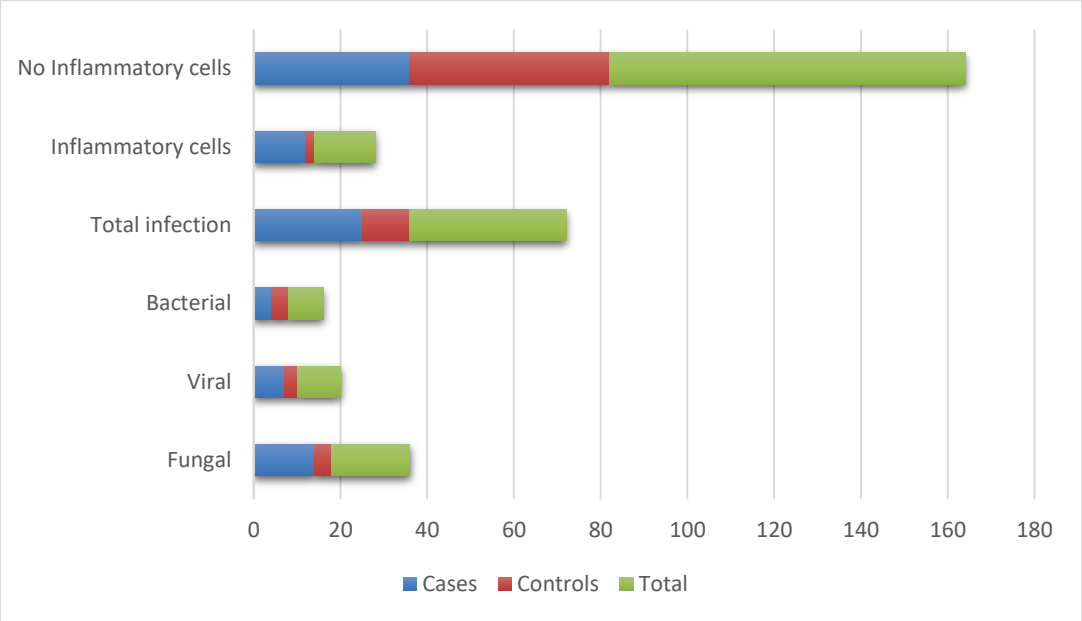


Figure 3 Cases and controls by infections and inflammatory cells infiltrate.

4. DISCUSSION

Early detection represents a vital health priority, which requires combined efforts, including screening at-risk populations and raising population awareness, which was reported to be relatively low in Saudi Arabia (Alrashdi et al., 2020). In Saudi Arabia, Smokeless tobacco use is common among residents rather than civilians (Alreshidi et al., 2017). Since there is a need to establish an oral cancer screening program, the present investigation was carried out to assess the merit of combined use of AgNORs and oral mucosal keratinization for early prediction of cytological atypia with the use of smokeless tobacco. Our investigation indicated that the use of both methods together revealed superior novelty in terms of specificity and sensitivity. NORs quantification has been widely used to denote cellular proliferative activity, which might proceed to cytological atypia and eventual malignant progression (Monti Hughes et al., 2020). However, AgNORs quantification was reported to have an average value in reporting some potential oral lesions, such as leukoplakia (de Camargo et al., 2020).

In the current study of oral epithelium cells, keratinization was identified in a high number of cases compared to the controls. Presence of keratinized epithelium cells in the cytological smears prepared from buccal mucosa, usually associated with exposure to chemical or mechanical stress indicating the progressive change (Nikoloudaki et al., 2020). Therefore, keratinization might be a useful marker for indicating oral mucosal injury. Both AgNORs quantification and keratinization as a qualitative measure were previously used to screen oral cavity-associated cellular proliferative activity in numerous studies. AgNORs count was used in a study to assess the effect of a type of smokeless tobacco known as "Toombak," and it was found useful for detecting oral mucosal lesions (Ahmed and Babiker, 2009).

These findings show that Saudi Arabia's smokeless tobacco is a significant risk for inducing oral mucosal proliferative activity. As many of the present study participants were Sudanese, all of them were using Toombak, which was previously reported as containing a high concentration of tobacco-specific nitrosamine, which was reported to be a potent carcinogen (Ahmed, 2013; Anass and Ahmed, 2013).

The present study's findings have shown high frequencies of infections and inflammatory cell infiltrates, which might be associated with the increased pattern of keratinization. Such assumption was previously reported (Sakaguchi et al., 2021).

5. CONCLUSION

The combined use of AgNORs and oral mucosal keratinization is useful for the early prediction of cytological atypia associated with smokeless tobacco use. Oral use of smokeless can cause oral mucosal keratinization and cellular proliferation.

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Author Contributions

HGA: Conception, administration, analysis, drafting, approval of the final version.
ABE; NKB; GMOE; RAHA; MAH; MABIB, EAAA: Conception, design, data acquisition, approval of the Final version
HS: Consultation, analysis, drafting, approval of the final version.

Ethical approval

All procedures performed in studies involving human participants were following the institutional and/or national research committee's ethical standards and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical approval number is HREC00121a/CM-UOH.04/20.

Informed consent

Informed consent was obtained from all individual participants for whom identifying information is included in this manuscript.

Data and materials availability

All data associated with this study are present in the paper.

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